



**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, DC 20460**

OFFICE OF  
PREVENTION,  
PESTICIDES  
AND TOXIC  
SUBSTANCES

April 15, 2011

**MEMORANDUM**

Subject: Efficacy Review for EPA Reg. No. 1677-EGU, DLSB-99;  
DP Barcode: 383470

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Applicant: Ecolab, Inc.,  
370 Wabasha Street North  
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**Formulation from the Label:**

<u>Active Ingredient(s)</u>	<u>% by wt.</u>
LAS.....	1.23%
Lactic Acid.....	17.29%
<u>Other Ingredients</u> .....	<u>81.48%</u>
Total.....	100.00%

## I BACKGROUND

The product, DLSB-99 (EPA File Symbol 1677-EGU), is a new product. The product is intended to control the growth of spoilage and decay causing organisms in the processing water of fruits and vegetables. Efficacy data was generated at Ecolab, located on the Ecolab Schuman Campus, on 655 Lone Oak Drive, in Eagan, MN 55121-1560. The current data package contained a letter from the registrant (dated September 30, 2010), two efficacy studies (MRID Nos. 482535-10 and -11), Statement of No Data Confidentiality for both studies, and the proposed label.

## II USE DIRECTIONS

According to the proposed label, the product is a water additive for pathogen reduction in fruit and vegetable process waters. Directions on the proposed label provided the following instructions for the preparation and use of the product:

Select a minimum exposure time and dilute product into the processing vessel according to the table below:

Minimum Contact Time	Ounces of concentrate per gallon of water	Dilution ratio (parts concentrate :parts water)	Active Ingredients	
			ppm LAS	ppm Lactic Acid
90 seconds	0.75 –1.25	1:170 – 1:100	76-139	1061-1953
5 minutes	0.5 – 0.74	1:256 –1:173	50-82	707-1156

## III AGENCY STANDARDS FOR PROPOSED CLAIMS

### Efficacy of Antimicrobial Agents to Reduce Foodborne Pathogenic Bacteria in Processing Waters for Fruits and Vegetables)

Antimicrobial agents are often added to water used in the transportation and/or washing of fresh fruits and vegetables to reduce the population of microorganisms in fruit and vegetable process waters. The test system used for this method includes three strains of *Listeria monocytogenes* (ATCC 49594, 1914, 19116), three strains of *Escherichia coli* serotype O157:H7 (ATCC 43895, 35150, 43890), and three strains of *Salmonella choleraesuis* (enterica) subsp. *choleraesuis* (serotype javiana ATCC 10721, serotype newport ATCC 6962, and serotype typhimurium ATCC 13311) be combined in equal volumes, with subsequent adjust to yield approximately  $10^{10}$  organisms per milliliter. A volume of 99 ml of the antimicrobial agent working solution is dispensed into a sterile 250 ml flask. Vigorously swirl flask, while immersing the tip of a pipet containing 1 ml of the test system suspension into the test solution midway between the center and edge of the flask. Dispense 1 ml of the test system suspension into 99 ml of the test solution. After a **1-2 minute exposure period**, transfer 1 ml of the test solution into 9 ml of inactivating agent using a sterile pipet and vortex to mix. This tube is considered a  $10^{-1}$  dilution of the test solution. Plate antimicrobial working solution test samples in duplicate 1 ml and 0.1 ml from the  $10^{-1}$  inactivating agent tube. In addition, prepare a

10<sup>-3</sup> dilution in PBDW and plate in duplicate 1 ml and 0.1 ml from this for *Listeria* test samples only. For the inoculum population tests, prepare 10<sup>-5</sup> and 10<sup>-7</sup> dilution in PBDW. Use pour plate technique with molten, tempered (46±2°C) Tryptic Soy Agar (or Brain Heart Infusion Agar for *Listeria monocytogenes*). After agar solidifies in the Petri plates, invert and incubate at 35±2°C for 48±4 hours. The antimicrobial agent is effective in reducing foodborne pathogenic bacteria in fruit and vegetable processing waters if the population of all test bacteria are reduced by >99.9% relative to the inoculum populations.

#### IV SYNOPSIS OF SUBMITTED EFFICACY STUDIES

**1. MRID No. 482535-10, “DLSB-99 Efficacy at 0.5 oz/gallon to Reduce Foodborne Pathogenic Bacteria in Processing Water for Fruit and Vegetables” by Laurinda Holen. Study Completion Date—September 15, 2010. Study Identification Number—1000050.**

This study was conducted against *Listeria monocytogenes* (ATCC 49594, 19114, and 19116), *Escherichia coli* O157:H7 (ATCC 43895, 35150, and 43890), and *Salmonella enterica* (ATCC 10721, 6962, and 13311). Three lots (Lot Nos. P012701, P032301, and P032401) of the product, DLSB-99, were tested according to the Test Method for Efficacy of Antimicrobial Agents to Reduce Foodborne Pathogenic Bacteria in Processing Waters for Fruits and Vegetables (protocol attached). Efficacy data was generated in the presence of 1% vegetable soil load prepared by blending 5 grams of tomatoes, 5 grams of carrots, and 5 grams of Iceberg lettuce. The test substance was diluted 0.5 oz/ gallon of 400 ppm hard water. Following incubation the growth from the French slants was harvested by adding 3 mL of phosphate buffered dilution water and sterile glass beads to each bottle. Growth was washed from the French slants and transferred to a sterile centrifuged tube including the glass beads used to harvest the culture. The test system suspensions were filtered through sterile Whatman No. 2 filter paper pre-wet with 1 ml phosphate buffered dilution water. The test system suspensions were collected in sterile test tubes and vortex mixed. Equal volumes of each strain of *Listeria monocytogenes* was combined in a sterile centrifuge tube. The same was done for *Escherichia coli* O157:H7 and *Salmonella enterica*. Ninety-nine (99) ml of the diluted test substance was dispensed in 250 mL flasks in triplicate and placed into a 25±2°C water bath. Following temperature equilibrate of ≥ 20 minutes, a test flask was whirled and 1.0 ml of the test system suspension was added midway between the side and the center of the flask slightly immersed in the test substance, without touching the sides. After a 5 minute exposure, a 1.0 ml portion of the test substance/test system mixture was transferred to a 9 ml neutralizer blank and mixed. Aliquots of 1.0 ml and 0.1 ml from the neutralizer tube were then pour plated in duplicate using the subculture medium. Controls included those for initial numbers, neutralization confirmation, sterility, and purity.

Note—The protocol was amended to allow for the repeat efficacy testing of DLSB-99 batch P012701 against the three strains of *Salmonella* due to contamination and inconsistent test results.

**2. MRID No. 482535-11, “DLSB-99 Efficacy at 0.75 oz/gallon to Reduce Foodborne Pathogenic Bacteria in Processing Water for Fruit and Vegetables” by Laurinda Holen. Study Completion Date—September 15, 2010. Study Identification Number—1000040.**

This study was conducted against *Listeria monocytogenes* (ATCC 49594, 19114, and 19116), *Escherichia coli* O157:H7 (ATCC 43895, 35150, and 43890), and *Salmonella enterica* (ATCC 10721, 6962, and 13311). Three lots (Lot Nos. P012701, P032301, and P032401) of the product, DLSB-99, were tested according to the Test Method for Efficacy of Antimicrobial Agents to Reduce Foodborne Pathogenic Bacteria in Processing Waters for Fruits and Vegetables (protocol attached). Efficacy data was generated in the presence of 1% vegetable soil load prepared by blending 5 grams of tomatoes, 5 grams of carrots, and 5 grams of Iceberg lettuce. The test substance was diluted 0.75 oz/ gallon of 400 ppm hard water. Following incubation the growth from the French slants was harvested by adding 3 mL of phosphate buffered dilution water and sterile glass beads to each bottle. Growth was washed from the French slants and transferred to a sterile centrifuged tube including the glass beads used to harvest the culture. The test system suspensions were filtered through sterile Whatman No. 2 filter paper pre-wet with 1 ml phosphate buffered dilution water. The test system suspensions were collected in sterile test tubes and vortex mixed. Equal volumes of each strain of *Listeria monocytogenes* was combined in a sterile centrifuge tube. The same was done for *Escherichia coli* O157:H7 and *Salmonella enterica*. Ninety-nine (99) ml of the diluted test substance was dispensed in 250 mL flasks in triplicate and placed into a 25±2°C water bath. Following temperature equilibrate of ≥ 20 minutes, a test flask was whirled and 1.0 ml of the test system suspension was added midway between the side and the center of the flask slightly immersed in the test substance, without touching the sides. After a 90 second exposure, a 1.0 ml portion of the test substance/test system mixture was transferred to a 9 ml neutralizer blank and mixed. Aliquots of 1.0 ml and 0.1 ml from the neutralizer tube were then pour plated in duplicate using the subculture medium. Controls included those for initial numbers, neutralization confirmation, sterility, and purity.

Note—The protocol was amended to allow for the repeat efficacy testing of DLSB-99 batch P012701 against the three strains of *Salmonella* due to contamination and inconsistent test results.

## V RESULTS

MRID No. 482535-10

*Listeria monocytogenes*

DLSB-99 Batch Replication	Survivors CFU/Plate from 1.0 ml of 10 <sup>-1</sup> Dilution (10 <sup>-1</sup> )	Survivors CFU/Plate from 0.1 ml of 10 <sup>-1</sup> Dilution (10 <sup>-2</sup> )	Average CFU/ml of Test Survivors	Log Reduction
P012701	0, 0	0, 0	<1.7 x 10 <sup>1</sup>	>6.92
	0, 0	0, 0		
	0, 0	1, 0		
P032301	0, 0	0, 0	<1.0 x 10 <sup>1</sup>	>7.15
	0, 0	0, 0		
	0, 0	0, 0		
P032401	0, 0	0, 0	<1.6 x 10 <sup>1</sup>	>5.94
	0, 0	0, 0		
	46, 43	3, 2		

*Salmonella enterica*

DLSB-99 Batch Replication	Survivors CFU/Plate from 1.0 ml of 10 <sup>-1</sup> Dilution (10 <sup>-1</sup> )	Survivors CFU/Plate from 0.1 ml of 10 <sup>-1</sup> Dilution (10 <sup>-2</sup> )	Average CFU/ml of Test Survivors	Log Reduction
P032301	0, 0	0, 0	<1.6 x 10 <sup>2</sup>	>6.72
	47, 43	4, 4		
	0, 2	0, 0		
P032401	2, 1	0, 0	<5.7 x 10 <sup>1</sup>	>6.89
	1, 0	0, 0		
	28, 0	20, 0		
P012701 (8/6/10)	77, 80	8, 17	< 4.4 x 10 <sup>2</sup>	>5.29
	52, 53	5, 4		
	0, 0	0, 0		
P032301	4, 5	0, 0	< 2.2 x 10 <sup>1</sup>	>6.59
	1, 0	0, 0		
	0, 0	0, 0		
P32401	20, 20	1, 0	<9.0 x 10 <sup>1</sup>	>5.98
	0, 1	0, 0		
	5, 7	1, 0		

*E. coli* O157:H7

DLSB-99 Batch Replication	Survivors CFU/Plate from 1.0 ml of 10 <sup>-1</sup> Dilution (10 <sup>-1</sup> )	Survivors CFU/Plate from 0.1 ml of 10 <sup>-1</sup> Dilution (10 <sup>-2</sup> )	Average CFU/ml of Test Survivors	Log Reduction
P012701	231, 153	19, 26	2.8 x 10 <sup>3</sup>	4.63
	179, 162	18, 20		
	TNTC, TNTC	51, 43		
P032301	TNTC, TNTC	37, 38	4.9 x 10 <sup>3</sup>	4.39
	TNTC, TNTC	53, 42		
	TNTC, TNTC	77, 46		
P032401	127, 106	32, 39	4.4 x 10 <sup>3</sup>	4.44
	192, 248	36, 46		
	168, 158	59, 50		

MRID No. 482535-11

*Listeria monocytogenes*

DLSB-99 Batch Replication	Survivors CFU/Plate from 1.0 ml of 10 <sup>-1</sup> Dilution (10 <sup>-1</sup> )	Survivors CFU/Plate from 0.1 ml of 10 <sup>-1</sup> Dilution (10 <sup>-2</sup> )	Average CFU/ml of Test Survivors	Log Reduction
P012701	0, 0	0, 0	<1.5 x 10 <sup>1</sup>	>6.97
	0, 0	0, 0		
	4, 0	1, 0		
P032301	0, 0	0, 0	<1.0 x 10 <sup>1</sup>	>7.15
	0, 0	0, 0		
	0, 0	0, 0		
P032401	1, 0	0, 0	<4.0 x 10 <sup>1</sup>	>6.89
	0, 0	0, 0		
	0, 19	0, 0		

*Salmonella enterica*

DLSB-99 Batch Replication	Survivors CFU/Plate from 1.0 ml of 10 <sup>-1</sup> Dilution (10 <sup>-1</sup> )	Survivors CFU/Plate from 0.1 ml of 10 <sup>-1</sup> Dilution (10 <sup>-2</sup> )	Average CFU/ml of Test Survivors	Log Reduction
P032301	4, 1	0, 0	<1.5 x 10 <sup>1</sup>	>6.72
	1, 0	0, 0		
	0, 0	1, 0		
P032401	0, 0	0, 0	<1.0 x 10 <sup>1</sup>	>6.89
	0, 0	0, 0		
	0, 0	0, 0		
P012701 (8/4/10)	0, 0	0, 0	<1.3 x 10 <sup>1</sup>	>6.82
	0, 0	0, 0		
	1, 3	0, 0		

*E. coli* O157:H7

DLSB-99 Batch Replication	Survivors CFU/Plate from 1.0 ml of 10 <sup>-1</sup> Dilution (10 <sup>-1</sup> )	Survivors CFU/Plate from 0.1 ml of 10 <sup>-1</sup> Dilution (10 <sup>-2</sup> )	Average CFU/ml of Test Survivors	Log Reduction
P012701	TNTC, TNTC	26, 30	1.4 x 10 <sup>1</sup>	3.93
	TNTC, TNTC	384, 370		
	TNTC, TNTC	32, 26		
P032301	TNTC, TNTC	26, 30	2.8 x 10 <sup>1</sup>	4.63
	TNTC, TNTC	27, 24		
	TNTC, TNTC	29, 29		
P032401	127, 106	11, 17	1.7 x 10 <sup>1</sup>	4.85
	192, 248	15, 23		
	168, 158	15, 20		

## VI CONCLUSIONS

1. The submitted efficacy study (MRID Nos. 482535-10) does not support the use of the product, DLSB-99 (at the 0.5 oz/gallon use rate prepared in 400 ppm hard water) as an antimicrobial agent to reduce foodborne pathogenic bacteria in processing waters for fruits and vegetables against the following microorganisms on hard, non-porous surfaces for a contact time of 5 minutes in the presence of 1% vegetable soil.

*Escherichia coli* O157:H7 strains (ATCC Nos. 43890, 43895, 35150)

*Salmonella enterica* strains (ATCC Nos. 10721, 6962, 13311)

*Listeria monocytogenes* strains (ATCC Nos. 49594, 19114, 19116).

The contact time, 5 minutes, is in excess of the contact time specified in the Agency's accepted protocol (1-2 minutes).

Note—The Agency does not have a standard/acceptable claim for vegetable soil.

2. The submitted efficacy study (MRID Nos. 482535-10) supports the use of the product, DLSB-99 (at the 0.75 oz/gallon use rate when prepared in 400 ppm hard water), as an antimicrobial agent to reduce foodborne pathogenic bacteria in processing waters for fruits and vegetables against the following microorganisms on hard, non-porous surfaces for a contact time of 90 seconds in the presence of 1% vegetable soil.

*Escherichia coli* O157:H7 strains (ATCC Nos. 43890, 43895, 35150)  
*Salmonella enterica* strains (ATCC Nos. 10721, 6962, 13311)  
*Listeria monocytogenes* strains (ATCC Nos. 49594, 19114, 19116).

Note—The Agency does not have a standard/acceptable claim for vegetable soil.

## **VII RECOMMENDATIONS**

1. The proposed label claim is acceptable regarding the use of the product, DLSB-99, as an antimicrobial agent to reduce foodborne pathogenic bacteria (as detailed below) in processing waters for fruits and vegetables for a contact time of 90 seconds at the 0.75/gallon use rate when prepared in 400 ppm hard water.

*Escherichia coli* O157:H7 strains (ATCC Nos. 43890, 43895, 35150)  
*Salmonella enterica* strains (ATCC Nos. 10721, 6962, 13311)  
*Listeria monocytogenes* strains (ATCC Nos. 49594, 19114, 19116)

Acceptable efficacy data supports the proposed claim.

2. The proposed label claim is unacceptable regarding the use of the product, DLSB-99, as an antimicrobial agent to reduce foodborne pathogenic bacteria (as detailed below) in processing waters for fruits and vegetables for a contact time of 5 minutes at the 0.50/gallon use rate when prepared in 400 ppm hard water.

*Escherichia coli* O157:H7 strains (ATCC Nos. 43890, 43895, 35150)  
*Salmonella enterica* strains (ATCC Nos. 10721, 6962, 13311)  
*Listeria monocytogenes* strains (ATCC Nos. 49594, 19114, 19116)

The proposed contact time exceeds the accepted Agency standard.

3. On the proposed label, the correct ATCC Number for *Salmonella enterica* is ATCC 13311, not 1311.

4. On the proposed label/package insert, to reflect the correct use pattern of the proposed product. Briefly, remove the claim "Reduce 99.9% of *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella enterica* on the surfaces of freshly cut fruits and vegetables" from the proposed label. The public health claim is limited to processing water.



5. The list of vegetables and fruits claimed on the package insert must be limited to spoilage, non-public health organisms.